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– effects on foraging behaviour and production parameters

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- effekter på födosöksbeteende samt produktionsparametrar

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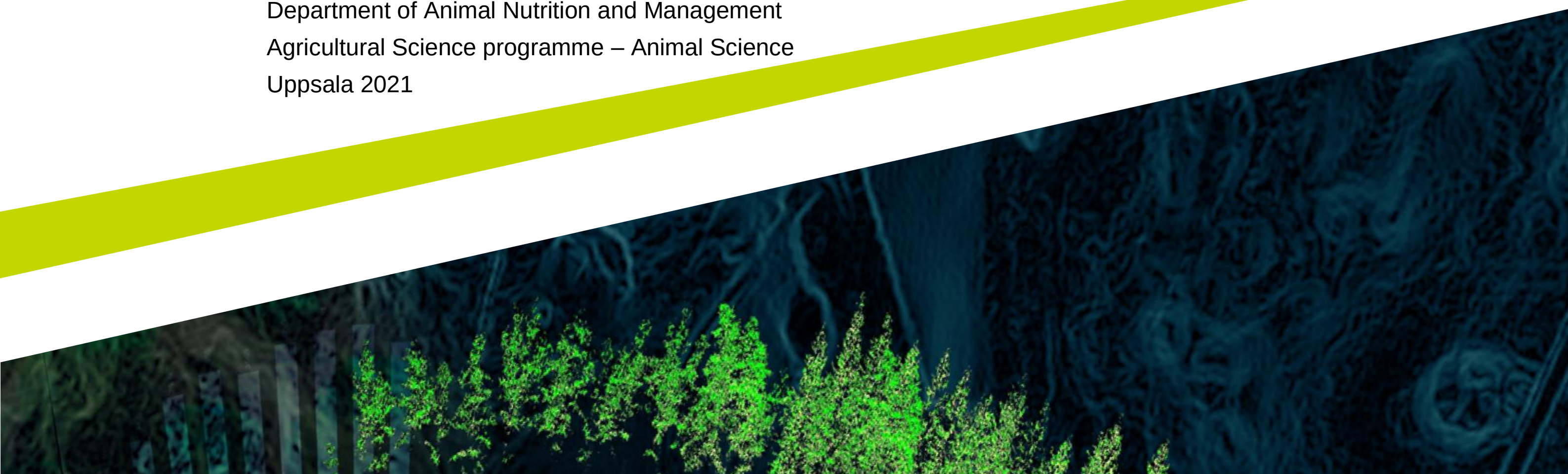

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Abstract

Feather pecking is a major welfare issue in commercial egg production. Feather pecking is thought to be a form of re-directed foraging pecking behaviour. When hens are deprived of the necessary conditions to perform foraging behaviour the risk of developing feather pecking increases. For this reason, promoting increased foraging behaviour has been used as a way to reduce feather pecking. There is some evidence that the provision of live Black soldier fly larvae can increase foraging behaviour in broilers and layers. However, it is not clear whether there are any differences between providing the larvae over a large versus a small area or for a longer time period versus all at once. For these reasons, the aim of this thesis was to investigate the effects of providing live Black soldier (20% of the daily nutritional need in DM) on foraging behaviour, growth and production in laying hens. The larvae were provided using one of three feeding methods: (1) with a bucket with holes (Bucket), hanging above the litter area, to provide larvae at a slow rate throughout the day, (2) larvae scattered on the litter area in the morning (Scatter) and (3) larvae provided in dedicated feeding troughs in the morning (Trough). A total of 90 laying hens were randomly assigned to one of the three treatments (6 pens per treatment, 5 hens per pen). Data was collected over 9 weeks and events of foraging, active behaviour, feather pecking and agonistic behaviour was registered during one day every other week using video cameras. On the day of recording, video cameras recorded each pen 1 hour before, the hour after and 5 hours after a provision of larvae which were delivered daily at 08.00 (standard time). Data for production parameters (feed consumption, egg production, egg weight, hen weight and weight of the intestine) were also collected throughout the study. The results showed that all methods managed to promote foraging when comparing the periods before and after a provision of larvae ($P < 0.05$). However, hens in the Bucket and Scatter treatments spent significantly more time foraging during the hour after a provision of larvae as compared to the hens receiving larvae in a trough ($P < 0.05$). Furthermore, hens in the Bucket treatment were more active compared to hens in the Trough treatment during the same period ($P < 0.05$). Both feather pecking and agonistic behaviour occurred too few times for any statistical analysis to be performed. All production parameters measured were unaffected by the feeding method. In conclusion, a provision of live black soldier fly larvae can increase the levels of foraging in laying hens and the method of providing it will affect the outcome. Methods where larvae is provided for a longer time period or being scattered over a larger area seems to be most advantageous compared to providing the larvae in troughs.

Keywords: laying hens, black soldier fly larvae, environmental enrichment, foraging, feather pecking

Sammanfattning

Förekomsten av fjäderplockning hos värphöns är ett stort djurvälfärdsproblem. Fjäderplockning tros vara ett slags omdirigerat födosöksbeteende. När värphöns inte erbjuds tillräckliga möjligheter att utföra födosöksbeteende ökar risken för förekomst av fjäderplockning. Främjandet av födosöksbeteende har därför blivit ett sätt att motverka förekomsten av fjäderplockning. Det finns viss bevisning för att en tilldelning av levande larver av Svart soldatfluga ökar förekomsten av födosöksbeteende hos slaktkyckling och höns. Det är dock oklart om effekten varierar beroende på om larverna sprids över ett större område jämfört med ett mindre eller under en längre tid jämfört med allt på en gång. Målet med detta arbete var därmed att undersöka födosöksbeteende hos värphöns som tilldelats levande larver av svart soldatfluga (20% av det dagliga näringsbehov i torrsubstans) genom tre olika metoder: (1) med hjälp av en Spann utrustad med hål (Spann), som hänger över ströbädden, för att larverna ska vara tillgängliga under en längre period, (2) larver utspridda över ströbädden på morgonen (Spridd) och (3) larver tilldelade i foderträg på morgonen (Tråg). Totalt inkluderades 90 värphöns vilka fördelades slumpmässigt mellan de tre behandlingarna (6 burar för vardera behandling, 5 höns i varje bur). Förekomsten av födosöksbeteende, aktivt beteende, fjäderplockning och agonistiska beteenden registrerades under 9 veckor (1 dag varannan vecka) med hjälp av videokameror. Videokameror spelade in 1 timme innan, timmen efter samt 5 timmar efter tilldelningen av larverna vilka gavs varje morgon 08.00 (normaltid). Data för produktionsparametrar (foderkonsumtion, äggproduktion, äggvikt, kroppsvikt samt vikt för utvalda inälvor) samlades också in under studien. Resultaten visade att samtliga tilldelningsmetoder främjade födosöksbeteende hos värphöns (jämförelse mellan perioden innan tilldelning av larver med perioderna efter; $P < 0.05$). Hönsen som tilldelades larver med metoderna kallade Spann och spridd utförde mer födosöksbeteende under perioden direkt efter tilldelning jämfört med hönsen som tilldelats larver i ett foderträg ($P < 0.05$). Vidare var hönsen i Spanngruppen mer aktiva jämfört med hönsen in tråggruppen under samma period ($P < 0.05$). Varken fjäderplockning eller agonistiska beteende kunde analyseras då dessa förekom i liten utsträckning. Inga av de studerade produktionsparametrarna påverkades av tilldelningsmetod. Sammanfattningsvis kan en tilldelning av levande larver av svart soldatfluga främja födosöksbeteende hos värphöns och metoden som används för att tilldela larverna har en inverkan. Tilldelningsmetoder där larver är tillgängliga under en längre tidsperiod eller utspridda över ett större område verkar vara mer fördelaktiga jämfört med att servera larver i ett tråg.

Nyckelord: *värphöns, larver av svart soldatfluga, miljöberikning, födosöksbeteende, fjäderplockning*

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Abbreviations

BSF	Black soldier fly
EE	Environmental enrichment
FP	Feather pecking

1. Introduction

Feather pecking (FP) is considered to be a major welfare issue within poultry production (Cronin & Glatz 2020) and must be dealt with in order to increase the sustainability of the production system. Its presence is problematic, not only because of the obvious suffering of the recipient but also because it indicates a frustration in the birds performing the FP behaviour (Blokhuis & Arkes 1984). Several studies have investigated FP behaviour in commercial layer breeds (*Gallus Gallus Domesticus*) and it is argued that the behaviour is closely connected to foraging behaviour (Blokhuis & Arkes 1984). Given their interdependence, science have sought ways of promoting foraging behaviour and reducing FP behaviour by introducing different enrichments into the housing of laying hens (hereafter referred to as hens; Dixon et al 2010).

The use of live insects, such as Black soldier fly (BSF; *Hermetia Illucens*) larvae, as an environmental enrichment (EE) for poultry is a growing field of interest. Insects have previously been investigated with the main goal of identifying new feed sources for production animals (Sogari et al. 2019). This research is highly motivated as some of the currently used feed sources, such as soybean, are considered unsustainable due to its connection with deforestation (Ermgassen et al. 2020). In addition to insects being an alternative and more sustainable feed source, researchers have begun to acknowledge its potential as an EE. To this date, only a few studies have investigated the behavioural effect on poultry following a provision of live BSF larvae but the results are promising (Ipema et al 2020; Star et al. 2020).

Going forward, it will be relevant to investigate different ways of providing the larvae to be able to identify a method which will promote foraging to the highest extent. Veldkamp & van Niekerk (2019) provided live BSF larvae to turkeys in troughs, once per day and found that the daily portion was consumed rapidly without any positive effect on foraging behaviour. Unlike Veldkamp & van Niekerk (2019), Ipema et al. (2020) did manage to promote foraging behaviour in broilers after providing live BSF larvae by scattering it and doing so several times per day. Star et al. (2020) studied a more gradual provisioning method of live BSF larvae for hens, however, they did not include foraging behaviour in their observations.

It is clear that there is a lack of knowledge regarding the effects on foraging behaviour in hens. Furthermore, there is a lack of comparisons between different methods of providing the larvae seeing as previous studies often studied one method and compared it to control groups not receiving any larvae at al. The identification and use of a method that promotes foraging to a high extent is arguably an important measure to counteract FP as hens will spend more time acting out a natural behaviour and thus may be less inclined to engage in FP.

Hence, the aim of this thesis was to investigate whether a provision of live BSF larvae would increase levels of foraging in hens and study the effects of providing the larvae over a larger versus a smaller area and for a longer time period versus providing it all at once. The hypotheses of this thesis are: 1) providing live BSF larvae during a 5h period will result in higher levels of foraging and active behaviour in hens than when provided with the larvae once a day (2) scattering live BSF larvae in the litter area will result in higher levels of foraging and active behaviour in hens than when provided with the larvae in a dedicated feed trough.

2. Literature review

2.1. Natural and abnormal behaviour

The process of animal domestication involves the adjustment to a life in captivity and the conditions, provided by man that comes with it (Price 1984). The genetic differences found in domesticated animals when compared with their ancestors are said to depend on three factors (Price & king 1968 see Price 1984). (1) The effect of natural selection, or perhaps better known as survival of the fittest, is less evident, (2) selection for specific trades linked to e.g. production is common and (3) there is often a lack of knowledge regarding the genetic linkage between selected trade and other, perhaps unwanted, trades. Despite the strong selection of production traits in domesticated animals, many behavioural needs persist even if their function is less relevant for artificial commercial environments.

These behavioural needs are often referred to as natural behaviours. A natural behaviour can be defined as a behaviour performed by animals under natural conditions as they are pleasing and/or stimulate biological function (Bracke & Hopster 2006). The inability to perform said behaviour might lead to poor animal welfare (Jensen & Toates 1993) and the development of abnormal behaviours (Blokhuis & Arkes 1984; Blokhuis 1986; Huber- Eicher & Wechsler 1997). Animals tend to express abnormal behaviours when they are kept under conditions which are lacking in one or several ways e.g. not enough litter, no social stimuli etcetera (Wiepkema 1984). Thus, it is important to plan the environment in which we keep our production animals, so that it promotes natural behaviour and prevents the development of abnormal behaviour as this is crucial for the animal welfare (Broom 1991).

2.1.1. Foraging

The Red Junglefowl (*Gallus gallus*), which is the main ancestor of the modern domesticated hen (Lawal et al. 2020), spends up to 90% of their active time foraging (Dawkins 1989). Poultry foraging behaviour is a collective name for activities such as ground-pecking, ground-scratching and grazing (Jensen 2017) and it is considered to be a natural behaviour. In studies comparing the Red Junglefowl and modern layer breeds, both types have been shown to spend a similar amount of their active time, around 30%, engaging in foraging activities (Schütz & Jensen 2001). However, studies have also shown that the general expression of the behaviour has changed somewhat. As an example, modern layer breeds which are given the opportunity to choose between feed that is easily accessible, provided in a trough, and feed that is scattered in litter, will consume a larger proportion of their daily intake from feed provided in a trough as compared to Red Junglefowl (Schütz & Jensen 2001). Andersson et al. (2001) further demonstrated differences in foraging between a semi-domesticated breed (Swedish Bantam) and its main ancestor. They found that a crossbreed between the Red junglefowl and Swedish Bantam exhibited a more energetically costly strategy as compared to the semi-domesticated breed. Hence, it would appear that the domesticated breeds have adapted slightly different foraging habits as compared to their ancestor.

Even though modern breeds tend to use a more energy conserving foraging strategy, the behaviour is still highly motivated. This was shown in a study by Bubier (1996) where hens were initially given free access to different pens with various enrichments such as woodchips, grass and perches. Each enrichment pen could be accessed from the barren middle pen. After some time, round wooden bars were mounted at the entrance of each enrichment pen to make them less accessible resulting in a decrease in the willingness of the hens to enter the pens. The behavioural data recorded during the study concluded that even though hens were less willing to access pens suitable for foraging, they would continue to perform foraging activities to the same extent. Duncan & Hughes (1972) provided further evidence supporting the notion that modern layer breeds are highly motivated to perform foraging behaviour. The hens in their study would continue to collect a portion of their daily intake from feeders requiring them to peck at a specific disc to gain access to the feed all while the same feed was freely available in an adjacent trough.

It is clear that foraging is a natural behaviour which modern layer breeds are highly motivated to perform (Schütz & Jensen 2001, Bubier 1996; Duncan & Hughes 1972), and the inability to perform said behaviour in a satisfactory way has been claimed to be one of the main reasons behind the development of FP (Blokhuis & Arkes 1984; Blokhuis 1986; Huber- Eicher & Wechsler 1997).

2.1.2. Feather pecking

Feather pecking is a non-aggressive abnormal behaviour observed in hens (Cronin & Glatz 2020) and occurs in one of two forms, gentle and severe (Savory 1995). Gentle FP is characterized by moderate pecking at the plumage of another bird. Generally, the recipient is left unharmed and the event does not result in any reaction performed by the receiver. The other form, severe FP, consists of violent pecking at another bird where the recipient's feathers may be pulled out in the process. Recipients have been shown to vocalize and/or attempt to avoid their attacker (Savory 1995; Hartcher et al. 2015). Eventually, severe FP may leave the receiver with large areas of bare skin (Savory 1995). Continued pecking at bare skin can cause haemorrhage, which may in turn attract even more pecks and, in extreme cases, evolve into cannibalism (Savory 1995). Cannibalism can be defined as pecking and pulling directed against already bare areas of skin (Keeling 1994) and the recipient may eventually die from its obtained injuries. In addition to the obvious discomfort FP inflicts, the loss of feathers will render a negative effect on feed conversion as the individual's ability to thermoregulate is compromised (Glatz 2001).

Feather pecking is thought to be a multifactorial issue and the identification of specific circumstances connected to the problem is a major research area where matters such as social environment, enrichment, nutrition and genetics have been studied (Rodenburg et al. 2013). However, redirected ground pecking due to suboptimal foraging opportunities is hypothesised to be one of the main underlying causes (Blokhuis & Arkes 1984; Blokhuis 1986; Huber- Eicher & Wechsler 1997). Indeed, studies have shown that there is a connection between the two behaviours, where hens that developed FP also engaged in high levels of foraging when young (Newberry et al. 2007). Furthermore, there is a connection between the age that hens gains access to litter material and hence opportunity to forage, and the prevalence of FP later on in life. Johnsen et al. (1998) showed that hens reared with access to both sand and straw from 1 day of age engaged in less FP as compared to hens reared without access even though both groups had access to sand and straw from 5 weeks of age. Tahamtani et al (2017) further showed that hens reared on paper, allowing droppings and feed spillage to remain in the pen and thus giving the hens the opportunity to forage among this, had better plumage condition at 30 weeks of age as compared to hens reared on mesh. This would indicate that there was less FP among the groups reared on paper.

Pending clear scientific advice on how to deal with the issue, the commercial egg industry has adopted beak trimming as a management strategy (Kuenzel 2007) as it has been shown that beak trimmed hens perform less severe FP (Gilani et al. 2013). Beak trimming is a partial removal of the beak and can be performed with a hot blade or through infrared treatment (Glatz & underwood 2020). However, this measure merely addresses the symptoms and not the actual root of the problem. Following the procedure, hens display a different behavioural pattern, with fewer pecks to the environment, as compared to individuals with intact beaks, indicating that the procedure is both acutely and chronically painful (Gentle et al. 1990). This practice is currently illegal in Sweden (Animal protection law 2018:1192, 4 chap. 2§) but still permitted within the European Union when deemed necessary (Council directive 1999/74/EC). Therefore, alternative solutions to reduce risk of feather pecking that do not involve mutilation are needed.

In 2015, Svenska Ägg, the Swedish industry organisation for egg producers, investigated the prevalence of FP in Sweden by interviewing egg producers (Svenska Ägg 2015). In total, 68 complete production cycles were included and producers estimated whether or not they had a problem with FP during the production period in question. Feather pecking appeared to be present in 25% of the included units and in 10%, it was considered to be a severe problem (Svenska Ägg 2015). In their own perception, ongoing FP was challenging to counteract and the main underlying cause was thought to be inaccurate lighting and/or faulty feed composition (Svenska Ägg 2015).

2.2. Environmental enrichment

Introducing some form of EE, such as objects or foraging material that help direct pecking behaviour to objects rather than pecking at other hens, have been proven successful at reducing FP (Blokhuis & Arkes 1984; McAdie et al. 2005; Dixon et al. 2010) and agonistic behaviour in hens (Johannson et al. 2016; Zepp et al. 2018). Furthermore, a lack of EE seem to render the opposite results with a higher prevalence of FP paired with a lower prevalence of foraging (Blokhuis & Arkes 1984). The clear interaction between the two behaviours validates the earlier mentioned theory, that FP is a type of redirected foraging behaviour (Blokhuis & Arkes 1984; Blokhuis 1986; Huber- Eicher & Wechsler 1997).

Environmental enrichment is a broad term which is applicable to anything that will improve the biological function in animals kept by humans e.g. production animals (Newberry 1995). According to Newberry (1995) an EE should be both relevant and functional for the category of animals in question. Thus, an EE intended to promote foraging activities in hens may benefit from being manipulable and/or edible. Both edible and inedible EE, such as wood shavings, straw, pecking string, plastic box, whole oats and silage, have been able to reduce the prevalence of FP in hens (Blokhuis & Arkes 1984; McAdie et al. 2005; Dixon et al. 2010; Johannson et al. 2016; Zepp et al. 2018). Indeed, producers in the previously mentioned survey conducted by Svenska Ägg, who did not experience any problems with FP stated that they continuously provided their hens with various edible and inedible objects (Svenska ägg 2015).

Inedible but manipulable objects, such as strings, have been successful at reducing feather pecking and hens remain interested in pecking at the string for almost two months (McAdie et al. 2005). However, pecking at strings does not yield a positive feedback to the hen in the form of nutrients and the level of engagement may therefore decrease over time. In nature, pecking objects would often result in food such as an insect. Therefore, providing an EE that also provides part of the diet nutrient requirements may provide a longer lasting and more efficient reduction of FP throughout the production cycle. Indeed, edible EE in the form of peanut butter, seeds and cabbage has been shown to reduce FP to a greater extent as compared to inedible EE such as wooden blocks (Dixon et al. 2010).

The effect of EE will also depend on when in life it is introduced and the duration of its availability (McAdie et al. 2005; Pichova et al. 2016). A comparison between the instant provision of a pecking string (1 day old) and a later provision (22 and 57 days old) showed that the prevalence of FP increased when EE was provided at a higher age (McAdie et al. 2005). There are however contradictive results where a late provision (180 days old) of litter material in the form of wood shavings have rendered the same results as provision from 1 day of age (Nicol et al. 2001). McAdie et al. (2005) further studied the effect of providing EE for a limited amount of time and found that even a short provision (4 hours each day) of a pecking string from an early age (1 day old) resulted in lower levels of FP. When using edible EE, the availability is naturally time limited seeing as the EE is eaten and therefore no longer available. Pichova et al. (2016) concluded that a provision of meal worms to broiler chicks only temporarily increased the levels of foraging and active behaviour and proposed the use of a device that would be able to provide meal worms at a slow rate and for a longer time period.

The design of such a device is crucial for the intended effect on FP and foraging. This is confirmed by Lindberg & Nicol (2001) who found that an EE in the form of an operant feeder (feed drops as hens peck at a specific part of the feeder), replacing ordinary feeders, actually increased the prevalence of FP. The authors speculated that this particular form of EE might have induced high levels of frustration, as hens were able to watch other hens receive feed from the operant feeder while they themselves may have been unable to access it without getting chased off.

Nevertheless, edible EE, provided at a slow rate and for a long time period may be a promising way of promoting foraging behaviour and reducing the prevalence of FP.

2.3. Black soldier fly

A number of studies have investigated the possibility to utilize insects as feed for production animals and BSF larvae have been pointed out as a promising future feed source for poultry (Abd El-Hack et al. 2020). Several studies have shown that poultry are willing to eat BSF larvae when included in a feed, in the form of meal (Gasco et al. 2019), when provided in a dried form (Ruhnke et al. 2018) and live (Star et al. 2020; Tahamtani et al. *submitted*). The nutritional composition of BSF larvae is characterized by a high content of both fat and protein which varies with different stages of life (Liu et al. 2017). The nutritional composition is also affected by the substrate used to rear the larvae (Barragan-Fonseca et al. 2017). A recent study have shown that important production parameters for hens such as egg quality, feed conversion ratio and mortality are unaffected by a provision of live BSF larvae (Star et al. 2020).

Apart from BSF larvae being a potential future feed source for poultry, the process of rearing it might increase the sustainability of the handling of waste streams in the future, as the larvae is able to ingest, grow and sustain a fitting nutrient composition when reared on organic waste products (Meneguz et al. 2018). Furthermore, BSF would not have to be imported from other countries as it is possible to produce and rear them locally. BSF is regarded as a resistant species but rearing is still advised to mimic that of other production animals when it comes to infection control (Joosten et al. 2020). Given that BSF larvae is a suitable feed source for poultry, it opens up the possibility to also utilize live BSF larvae as a functional and biologically relevant EE.

2.3.1. Live Black soldier fly larvae as environmental enrichment

Currently, only a few studies have investigated the utilization of live BSF larvae as a feed source for poultry (Gunawan et al. 2018; Ipema et al. 2020; Star et al. 2020; Veldkamp & van Niekerk 2019) and even fewer have looked at its effect on poultry behaviour. Veldkamp & van Niekerk (2019) hypothesized that the provision of live BSF larvae would increase foraging behaviour in turkeys and also decrease the prevalence of FP. Live BSF larvae, corresponding to 10% of the daily feed intake, were provided in a trough once per day in the late morning and behaviour was recorded using video cameras. Initially, turkeys receiving larvae exhibited more feed pecking as compared to control groups who did not receive any. No differences were found for ground pecking and object pecking during the same period. However, as the turkeys aged, control groups started exhibiting more foraging behaviour as compared to the treatment groups. Feather pecking occurred to the same extent in both treatment and control group. Furthermore, authors noted that agonistic behaviour occurred less in the treatment group as compared to the control group. Authors discussed that the provision of the live BSF larvae, once a day in a trough, may have been influential for their results and suggested that several provisions throughout the day might yield better results, seeing as the turkeys consumed the larvae within minutes.

Ipema et al. (2020) compared the effects of providing (scattered on the litter) different amounts (5% vs 10%) of live BSF larvae at two different intervals (2 vs 4 times per day) on foraging and active behaviour in broilers. The results showed that the group receiving a larger amount, 4 times per day tended to exhibit more foraging behaviour as compared to the other groups. However, all groups who received any amount of larvae, regardless of how many times per day they were provided, engaged in more foraging activities as compared to the control groups which did not receive larvae. Treatment groups were also more active in general as compared to the control group.

Star et al. (2020) further investigated another provision method, using a dispenser which would distribute live BSF larvae continuously, during a period of 6 hours. Plumage condition in the participating hens were recorded and compared between groups receiving larvae and control groups. The results showed that treatment groups had better plumage condition as compared to the control group indicating that there were a lower prevalence of FP in the treatment groups.

2.3.2. Legislation

The use of insects as feed for production animals such as hens, whose ancestors normally feeds of insects as a part of their everyday diet (Bump & Bohl 1961), might not seem that controversial. Nevertheless, it is currently not permitted within the European Union to use insects as feed for non-ruminant production animals except aquatic production animals (Commission regulation 56/2013). However, the use of insects as feed within aquaculture only became possible during the last decade (Commission regulation 56/2013) and one might speculate that other changes, applicable to other non-ruminant production animals will follow. Indeed, it only just recently became possible to distribute insects as human food within the European Union (Commission regulation 2015/2283), indicating that this is an area under reconstruction.

The general precaution towards feeding production animals with other animals or animal parts can partially be explained by the massive outbreak of Bovine-spongiform-encephalopathy (BSE) in United Kingdom, in the 1980s, causing the death of cattle and eventually the formation of a variant form of Creutzfeldt-Jakob disease in humans (Collee & Bradley 1997, Scott et al. 1999). At this time, it was not uncommon to use discarded fat and carcasses from other animals as feedstuffs for example cattle (Collee & Bradley 1997). The different ingredients were usually grinded and processed resulting in the end product often referred to as meat-and-bone meal (Collee & Bradley 1997). This product was later found to be the cause behind the outbreak of BSE as it carried the infective agent, derived from other ruminants (Collee & Bradley 1997).

3. Materials and methods

This study is a part of a larger project which aims to investigate the potential use of live BSF larvae as a novel EE to promote foraging behaviours and reduce FP in hens. A secondary aim of this project is to utilize locally grown larvae as an alternative feed source for poultry to reduce reliance on imported proteins.

3.1. Animals and housing

This study included 90 Bovans White non beak trimmed hens acquired from a commercial rearing farm (Närkesberg Hönseri AB, Åsbro, Sweden) at 15 weeks of age. Hens were loose housed during rearing and were given access to litter material at the age of 22-24 days. All hens had been vaccinated against infectious bronchitis, Marek's disease, Coccidiosis and avian encephalomyelitis upon arrival.

The hens were housed until 25 weeks of age in the experimental facilities at SLU, Lövsta. The room where they were kept was equipped with 18 identical pens (3m × 3.56m × 3.62m, H × W × L), organised in two rows facing a middle aisle. Each pen was equipped with perches, litter area (1.32m × 3.56m, W × L), slatted area (2.30m × 3.56m, W × L), nest boxes, feed dispenser, a bell drinker and wood shavings as litter. The temperature was kept at 21-24°C and the light schedule can be seen in figure 1. Light intensity was 10 Lux.

For this study, each pen housed five hens, resulting in a stocking density of less than 1 hen/m^2 which can be compared to the permitted stocking density of 9 hens/m^2 in this type of pen (2 kap. 12 § Swedish board of agriculture’s regulations and general advice on poultry farming etc. [SJVFS 2019:23] Casenr. L111). The hens were allowed 2 weeks of habituation to the experimental facilities, including live BSF larvae (a handful/pen) which were provided in two small troughs (22cm × 3.5cm, W × H) in each pen for 5 consecutive days followed by 2 days without prior to the start of the study, on week 17 of age.

Ethical statement: All procedures involving animals were approved by the ethical committee of the Swedish Board of Agriculture (Jordbruksverket), application number 5.8.18-03402/2020

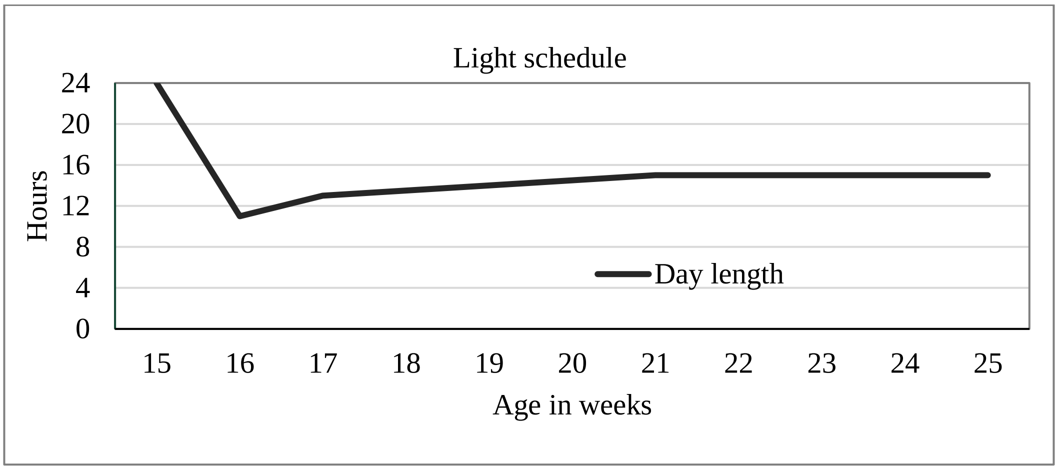

Figure 1. Light schedule for the habituation- and data collection period

3.2. Feed

A commercial pellet crush feed was provided ad libitum in two feed dispensers in each individual pen. The nutritional composition of the pellet crush feed, as provided by the manufacturer, can be seen in table 1.

3.2.1. Black soldier fly larvae production

Live BSF larvae, corresponding to 20% of the hens daily nutritional need in dry matter (62.5g/hen/day) were provided during the data collection period. The BSF larvae were grown at the colony of the Environmental Engineering group at the Department of Energy and Technology, SLU, Uppsala.

The production of the larvae took place in an adapted shipping container during the months of February-April, with an average temperature of 27°C and a relative humidity of 40%. The following feeding regime to produce the BSF larvae were used: The starter larvae (1 mg/larva) were reared in boxes (60cm × 40cm × 20cm) and kept in racks of 11 boxes. Each box contained 15,000 larvae which equals to 6 larva/cm^2. The applied feed was calculated so each larva received 0.2 g volatile substance/larva of poultry feed throughout the growth period. The feed pellets were watered down with 1:2 parts of water to achieve a feed containing 30% dry matter. The feed provided to the larvae was poultry feed left-over from SLU's experimental poultry farm at Lövsta. The feeding was split into 10 feedings during the larval growth period with the larvae receiving 1 kg/day. The larvae were harvested just before they turned into prepupa.

The live larvae were then packed in plastic boxes (4cm ×12cm × 17.5cm, H × W × L; 500ml; art nr F500, Tingstad) equipped with airholes and delivered to the research stable (Lövsta) two times per week during the data collection period. The content of two plastic boxes were equivalent to the daily portion for one pen. At Lövsta, the live BSF larvae were stored in a cold room (15°C) 1-4 days before being used.

Table 1. Composition of pellet crush feed

Nutrient concentration (g/kg[1])	Content
Energy (MJ)	11.2
Crude protein	155
Lysin	7.0
Methionine	4.0
Methionine + Cysteine	7.0
Calcium	37
Phosphorous	4.1
Sodium	1.5
Chloride	2.0

[1]Unless another unit is stated.

3.3. Experimental treatments

Hens were randomly assigned to one of the 18 pens (5 hens per pen, 6 pens per treatment): (1) larvae scattered on the litter at a slow rate throughout the day using a bucket with holes hanging above the litter area (Bucket), (2) larvae scattered on the litter in the morning (Scatter) and (3) larvae provided in troughs in the morning (Trough). Pen-treatment allocation was randomized throughout the building (figure 2). The starting weight of the hens in each treatment were not significantly different at the beginning of the study (Bucket LS Means ± SE: 1.25 ± 0.02kg; Scatter LS Means ± SE: 1.27 ± 0.02kg; Trough LS Means ± SE: 1.23 ± 0.02kg). For exact analysis, see 3.6.2.

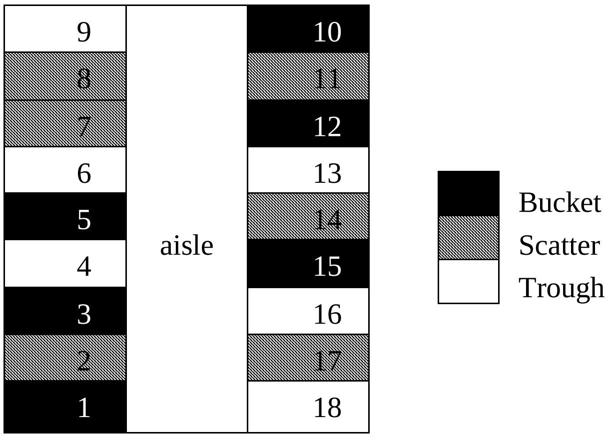

Figure 2. Respective treatment for each pen

The buckets (26cm × 22cm, H × W) used in the Bucket treatment were suspended above the litter area at an approximate height of 3m. Initially, the buckets were equipped with 33 holes, equally distributed over the bottom part. However, during the second and third week of the study, another 104 and 30 holes, respectively were added to all buckets as the larvae did not vacate as expected. Piloting beforehand had shown that at least 75% of the larvae would vacate the buckets within 5 hours but this was not the case when left-over larvae were weighed, approximately 6-8 hours after feeding. The average amount of larvae left in the buckets during the second and third week were 189g and 126g respectively which meant that only 30% and 60% of the larvae had vacated the buckets after 6-8 hours.

A second bucket (26cm × 20cm, H × W), with 121 holes, was installed in the pens assigned to the Bucket treatment during the third week. This allowed the portion of larvae to be divided between two buckets in each pen and was meant to increase the vacating speed of the larvae. The height of the buckets were also altered during this week to approximately 2m above the litter area to increase the amount of light reaching the inside of the buckets, again, as a measure of stimulating the larvae to vacate the buckets. White bike lights were also installed during the third week with the same intent but were removed by the end of this week as it had no effect.

In another attempt to make the larvae vacate the buckets, the daily portion of larvae for each pen were placed in a separate, smaller bucket (22cm × 16cm, H × W) the day before they were used, giving the larvae more space. Previously, the larvae were kept in a small plastic box (4cm × 9cm × 15cm, H × W × L) up until the day they were being used. This measure, together with the extra holes and bucket in each pen worked and by the end of the third week there was an average of 9g larvae (3%) left in the buckets in each pen after 6-8 hours. All larvae, regardless of treatment were placed in small buckets one day before they were used from the third week of the study and onward.

A final measurement of the vacating rate, performed by the end of the study, confirmed that approximately 75% of the larvae had indeed vacated the bucket within 5 hours.

3.4. Behavioural scoring

Video cameras were installed and set up to record the behaviour 1 hour before, the hour after and 5 hours after the provision of live BSF larvae which were provided daily at 08.00, standard time (figure 3; figure 4). The three recording sessions were referred to as periods with period 1 (P1) being the period before larvae were provided, period 2 (P2) being the first period after the larvae were provided and period 3 (P3) being the period 5 hours after larvae were provided. Each pen was recorded once per week. However, due to lack of sufficient cameras, the recordings took place during two consecutive days, with half of the pens being recorded the first day and the other half during the next. The recordings during these days were balanced with an equal number of each treatment being recorded each day.

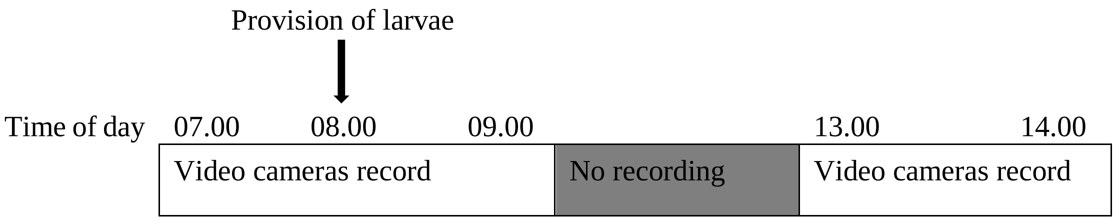

Figure 3. Schedule of recordings, each pen was recorded between 07.00-09.00 and 13.00-14.00 during one day each week throughout the study.

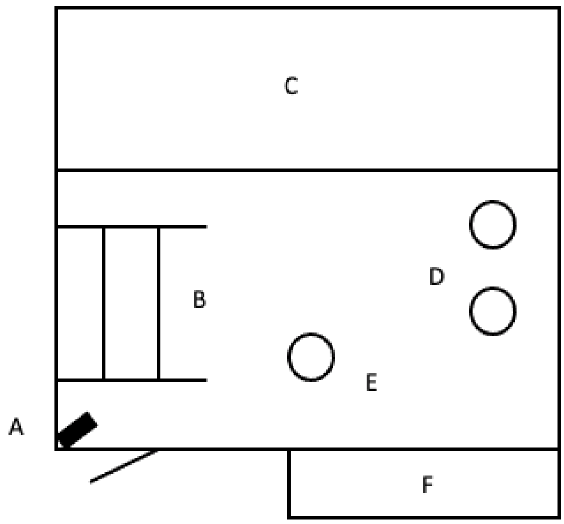

Figure 4. Schematic of pen with video camera (A), perches (B), litter area (C) which is where the larvae was provided, feed dispenser (D), bell drinker (E) and nest boxes (F).

Recordings were scored using the Observer XT software version 14 by two observers and both treatment and day of recording were balanced between them. The inter-observer reliability was evaluated prior to the scoring and deemed to be sufficient (Cohen´s kappa > 0.8). A scan sampling method was used to record the behaviour of the hens. In total, each scored hour included 55 scans resulting in a scan interval of 65.5 seconds. The behaviour of all five hens were recorded each scan resulting in one registered behaviour per hen each scan. In order to be able to establish the behaviour, hens were observed up to 10 seconds during each scan. In this thesis, video recordings from every other week of the data collection period were included resulting in 216 recorded hours. Hens were not individually marked. The ethogram used to score each behaviour can be seen in table 2.

Table 2. Ethogram

Behaviour	Description
Resting	Sitting or lying while not engaged in other activities. Resting on the ground, not standing on both feet.
Perching	Standing or sitting on the available perches.
Standing	Standing on the ground with both feet.
Locomotion	Running, walking, jumping, hoping or flying without performing any other type of behaviour.
Comfort Behaviour	Preening (manipulating own plumage, including pauses between each beak-feather contact), wing flapping, stretching, feather ruffling, and dustbathing.
Feather pecking	Pecking or pulling at the feathers of another individual. Includes the pauses between each peck, which often involves following the recipient hen. Includes both gentle and severe FP.
Agonistic behaviour	Hopping towards another hen, performing frontal threats (the two hens involved in the encounter have an upright position towards each other), leaping, sprinting toward another hen. Kicking and wing-flapping can be added to the frontal threatening, as well as aggressive pecking (forcefully pecking directed toward the head (generally) of another hen - the peck either results in contact or causes an avoidance response/squat in the target hen).
Foraging	Pecking or scratching the litter area or the empty troughs.
Exploratory behaviour	Pecking at objects in the pen, e.g. walls, slats, perches, etc.
Eating	Consuming concentrated pellet crush feed at the assigned feeders.
Eating larvae	Consuming larvae from the troughs (only relevant to the Trough treatment).
Drinking	Consuming water at the bell drinkers.
Out of sight	Not visible in the recording, e.g. inside nest boxes.

3.5. Other data collection

3.5.1. Production parameters

Pellet crush feed consumption in each pen was recorded and registered on a weekly basis using a hand scale (accuracy 20g), weighing the entire feed dispenser (container + feed). The weight of the empty feed dispenser were collected by the end of the study and subtracted when calculating the feed consumption. Furthermore, eggs were collected daily and weighed once per week. Each hen was also individually weighed upon arrival at 15 weeks of age, half way through the data collection period at 21 weeks of age and at the end of the study at 25 weeks of age. Hens were put in a plastic box, which was then placed on a scale. The scale had an accuracy of 10g and the weight of the plastic box was tared.

3.5.2. Post mortem assessment

By the end of the study, at 25 weeks of age, 12 animals from each treatment were euthanized and a post mortem assessment was performed where the weight of the gizzard (empty), proventriculus (empty), abdominal fat pad and liver were collected for each hen. The hens were euthanized with an intravenous bolus injection of pentobarbital (Allfatal vet. 100mg/ml. Omnidea AB, Stockholm) and cut open using scalpels. Fat covering both gizzard and proventriculus was trimmed of and any feed residues were rinsed prior to the weighing.

3.6. Statistical analysis

3.6.1. Behaviour

For the purpose of this thesis the following behaviours were analysed: foraging, active behaviour, feather pecking and agonistic behaviour. Active behaviour was a summary of all behaviours except for resting, perching, standing, feather pecking and out of sight. Furthermore, foraging was a summary of both foraging and eating larvae for the Trough treatment. This was because the larvae were provided in the litter area and time spent eating larvae were therefore considered a part of the foraging behaviour.

Given that hens were not individually marked, behavioural data were summarized per pen and period. All behavioural data were analysed with the JMP Pro software version 15.2.1. The assumption of normality of the residuals were tested using a Shapiro-Wilk test and any data not passing the test were square root transformed to approximate normality (foraging and eating larvae). All variables were analysed using least square analysis with treatment, period and week of age as fixed effects as well as significant interaction between fixed effects. The model also included pen as a random effect. Post hoc analysis was performed with Tukey HSD test for multiple comparisons.

Feather pecking and agonistic behaviour was observed too few times for any individual statistical analysis to be performed which is why only descriptive data is presented in the results section.

3.6.2. Production parameters and post mortem assessment

All production parameters and organ weights were analysed with the JMP Pro software version 15.2.1. The assumption of normality of the residuals were tested using a Shapiro-Wilk test. Egg production and egg weight data was normalized by removing points considered outliers (defined as 1.5*IQR below Q1 and 1.5*IQR above Q3). Five outliers for egg weight (Bucket:2 Scatter:2 Trough:1) and 14 for egg production (Bucket:3 Scatter:5 Trough:6) were removed to approximate normality.

All production parameters were analysed using least square analysis with treatment and week of age as fixed effects as well as significant interaction between fixed effects. The model also included pen as a random effect. Post hoc analysis was performed with Tukey HSD test for multiple comparisons.

The weight of organs were also analysed using least square analysis with treatment as fixed effect and pen as a random effect. Body weight was added as a covariate. Post hoc analysis was performed with Tukey HSD test for multiple comparisons.

4. Results

Data in graphs are presented as means ± standard error exempt for FP and agonistic behaviour where only descriptive data is presented.

4.1. Behaviour

4.1.1. Foraging

There was a significant interaction between treatment and period of the day on foraging behaviour ($F_{4,189}$ = 7.33; P < 0.0001). Hens in the Bucket and Scatter treatments spent significantly more time foraging during period 2 than hens in the Trough treatment (P < 0.05; figure 5). At large, foraging behaviour was higher after a provision of larvae (P2 and P3) and lower during the period before (P1; P < 0.05). There was no effect of week of age ($F_{3,189}$ = 1.17; P < 0.32).

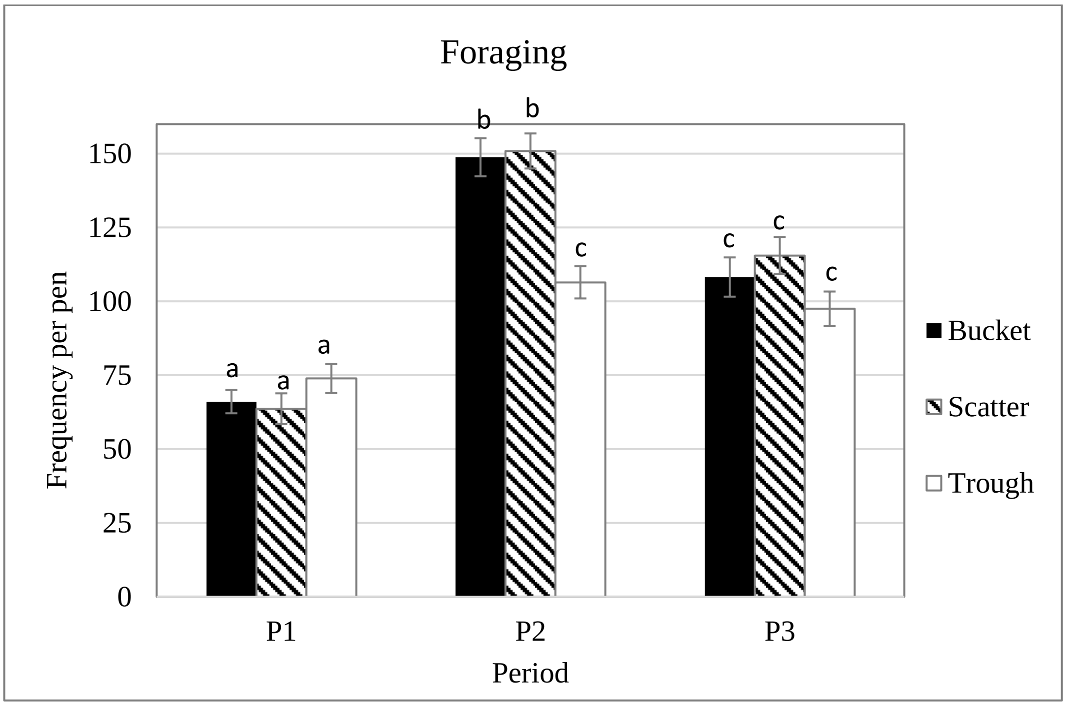

Figure 5. Frequency of foraging and eating larvae per pen, before (P1), during (P2) and after (P3) feeding live BSF larvae. Different superscript letters indicate significant differences.

4.1.2. Active behaviour

Again, there was a significant interaction between treatment and period of the day on the amount of time the hens spent engaging in active behaviours ($F_{4,189} = 5.30$; $P < 0.005$). Hens in the Bucket treatment spent significantly more time performing active behaviours during period 2 than hens in the Trough treatment during the same period ($P < 0.05$; figure 6). There was however no significant difference between the amount of active behaviour for hens in the Scatter treatment during the same period when compared with the other two treatments ($P < 0.05$). Hens in all treatments were in general more active after the provision of larvae, as compared to before (P1; $P < 0.05$). Meanwhile, there was no effect of week of age ($F_{3,189} = 0.85$; $P < 0.47$).

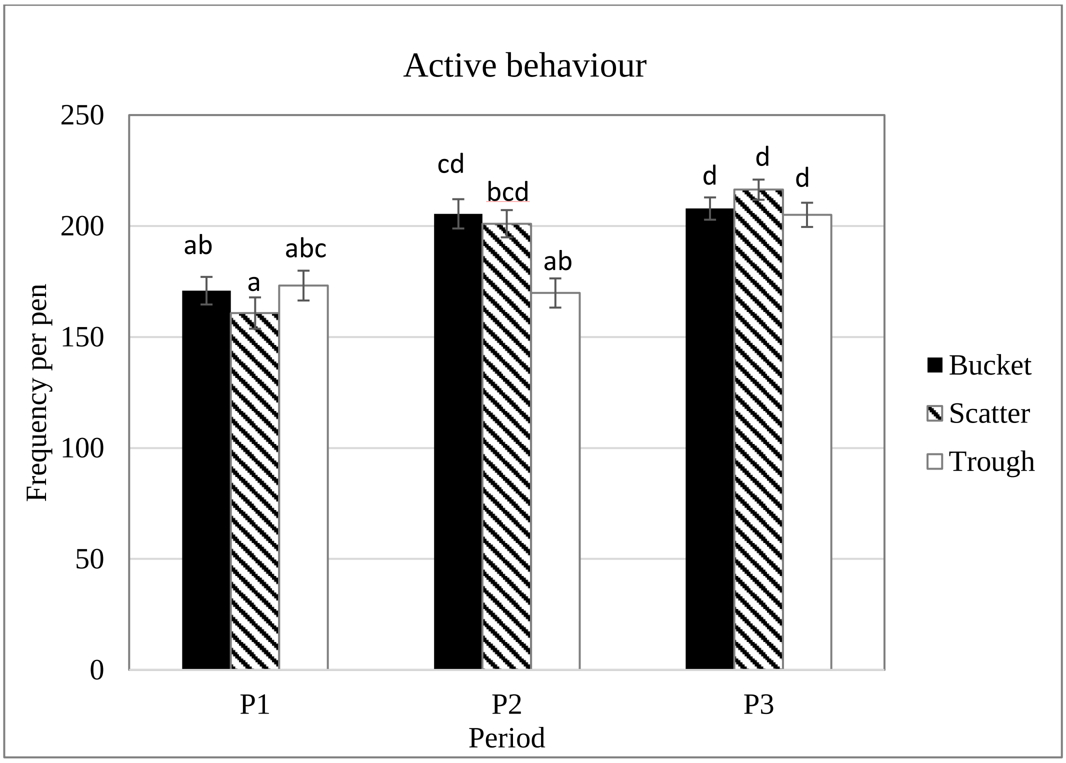

Figure 6. Frequency of active behaviour per pen, before (P1), during (P2) and after (P3) feeding live BSF larvae. Different superscript letters indicate significant differences.

4.1.3. Feather pecking

Feather pecking occurred at a low frequency, out of 59 400 individual observation points, FP was observed only in 29 occasions. Feather pecking was present in all treatments, the highest number of events occurred during the second period (P2) with the Bucket treatment accounting for the majority of FP events during this period (n=6, figure 7).

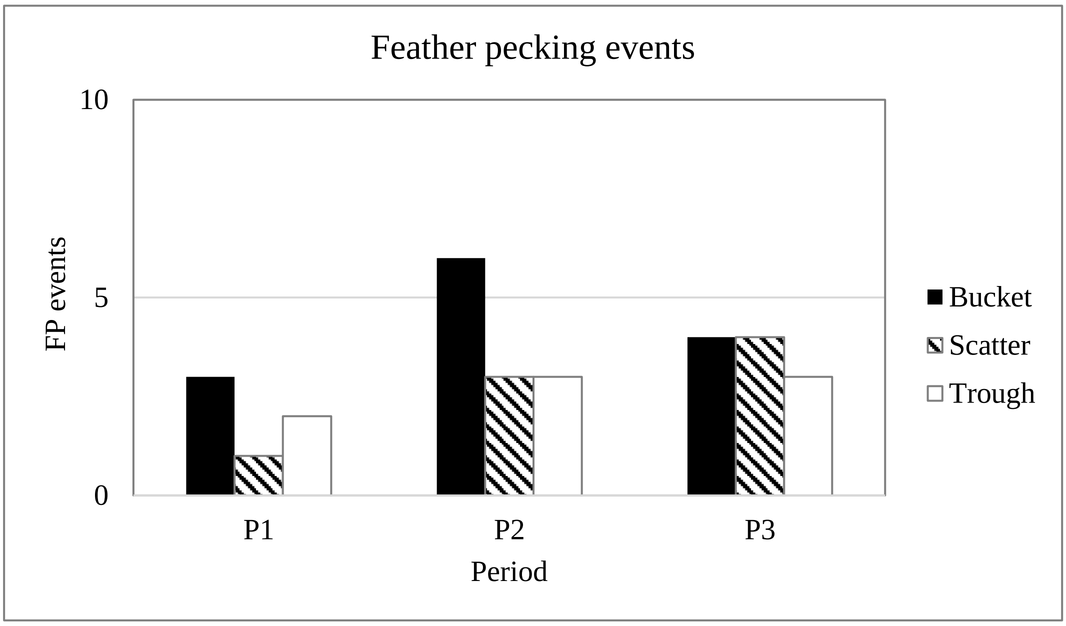

Figure 7. Total number of FP events registered during the entire study before (P1), during (P2) and after (P3) feeding live BSF larvae.

4.1.4. Agonistic behaviour

Much like FP, agonistic behaviour occurred at a very low frequency (15 times in total) with 2/3 of the agonistic behaviour observed in the Bucket treatment (during P1, P2 and P3, n=2, n=6 and n=2 respectively) and 1/3 observed in the Scatter treatment (during P1, P2 and P3, n=2, n=1 and n=2 respectively). No agonistic behaviour was observed in the Trough treatment.

4.2. Feed consumption

There was no effect of treatment on the feed consumption ($F_{2,15}$ = 1.45; P = 0.27). There was however an effect of week of age ($F_{8,136}$ = 4.34; P < 0.0001), with the feed consumption fluctuating over the weeks (figure 8).

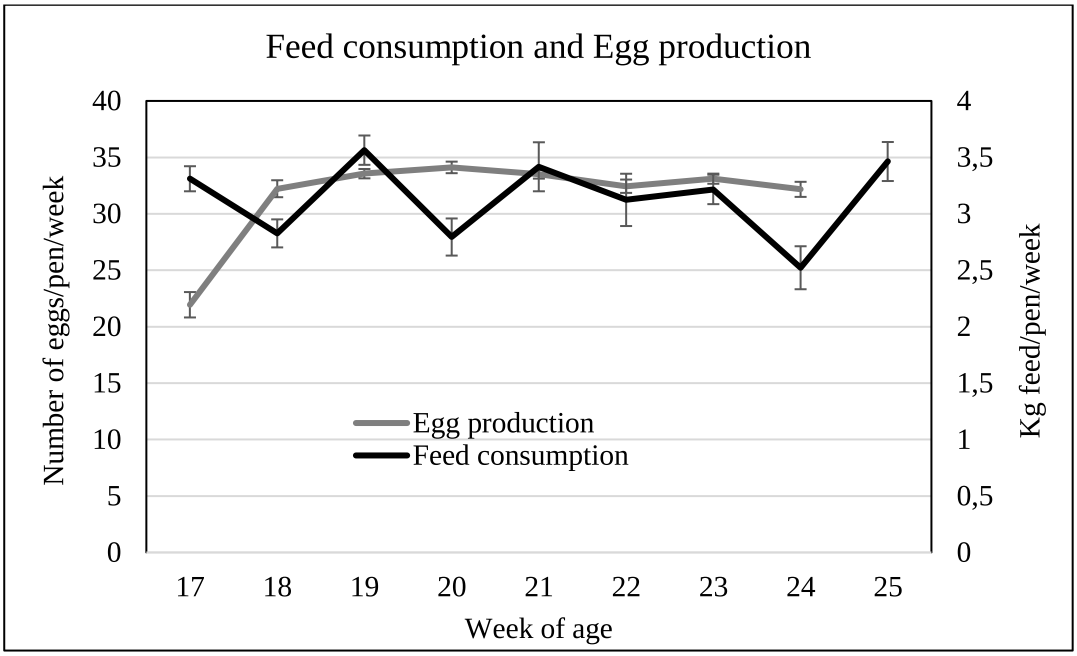

Figure 8. Feed consumption and egg production per pen for each week of age during the entire study.

4.3. Egg production and egg weight

Treatment had no effect on egg production ($F_{2,14}$ = 2.40; P = 0.12). There was, however, an effect of week of age ($F_{7,105}$ = 13.16; P < 0.0001), with the hens laying fewer eggs on week 17 compared to all other ages (P < 0.0001; figure 7). In regards to the weight of the eggs, there was no effect of treatment ($F_{2,14}$ = 0.70; P = 0.51). As expected, the weight of the eggs increased as the hens grew older, weighing approximately 47g on week 17 and 58g on week 25 ($F_{8,131}$ = 28.26; P < 0.0001).

4.4. Weight and post mortem assessment

Finally, there was no effect of treatment on the weight of the hens at any age ($F_{2,15}$ = 0.30; P = 0.75). There was however, an effect of week of age ($F_{2,250}$ = 371.6; P < 0.0001), with hens being significantly lighter at 15 weeks of age (LS Means ± SE: 1.25 ± 0.012kg) compared to at 21 and 25 weeks of age (LS Means ± SE: 1.57 ± 0.012 and 1.58 ± 0.012kg respectively; P < 0.0001). In regards to the liver, there was no effect of treatment and body weight ($F_{2,13}$ = 1.11; P = 0.36), with all livers weighting on average 50.67 ± 5.45g (means ± standard deviation). There was also no effect of treatment on the weight of the proventriculus ($F_{2,13}$ = 1.74; P = 0.21), with all the proventriculi weighing on average 6.44 ± 0.61g (means ± standard deviation). There was also no effect of treatment on the weight of the gizzard ($F_{2,14}$ = 0.57; P = 0.58; mean ± std dev: 31.52 ± 6.89g). Finally, there was no effect of treatment on the weight of the abdominal fat pads ($F_{2,14} = 0.08$; $P = 0.92$; means ± standard deviation = 41.88 ± 12.56g). The individual variation was however quite large with the lightest fat pad weighing 15g and the heaviest weighing 70g.

5. Discussion

This study aimed to investigate various methods of providing live BSF larvae to hens and identify the method which promoted foraging behaviour to the highest extent in hens. Larvae were provided live to encourage directing pecking behaviour towards search of food (i.e. foraging behaviour) rather than pecking at each other and thus reduce the risk of FP. The use of live BSF larvae as an environmental enrichment for poultry is still a rather new field and only a limited number of studies have investigated its use as an EE while looking at foraging behaviour. The studies have yielded varying results, with a higher frequency of foraging behaviour in broilers (Ipema et al. 2020) and a lower frequency in turkeys (Veldkamp & van Niekerk 2019) as compared to control groups not receiving any live BSF larvae. One distinctive difference between the mentioned studies were the provision methods and it is clear that a comparison between methods is lacking which is how this study has sought to contribute.

The results from this study suggest that provision methods where live BSF larvae are spread across a larger area (Scatter) or provided for an extended time period of at least five hours (Bucket) will promote foraging to a higher extent as compared to providing it all at once in a trough (Trough). Furthermore, the method referred to as Bucket will also promote active behaviours to a higher extent compared to the other methods. In regards to important production parameters such as feed consumption, egg production, egg weight, body weight and weight of the intestine, it would seem that they are unaffected by the method of provision.

5.1. Behaviour

All behavioural data in this study is based on manual video scoring performed by two observers. Pens and periods were balanced between the two to limit any observer effect and the inter-observer reliability was evaluated and deemed acceptable beforehand. However, the observers could not be blind to the treatment of the pens during video scoring due to the fact that the exact time of provision had to be identified for each pen as the starting point of observations. At that point it was obvious which treatment it was as the observer watched the personnel provide the larvae in different manners according to the treatment. Observers were also well aware of which treatments that were expected to yield higher levels of foraging behaviour which might have caused bias (Tuyttens et al. 2014). Arguably, the results would therefore have a better credence if observers were blinded which is recommended for future studies.

5.1.1. Foraging

Feeding larvae to hens by using a bucket to slowly release larvae or by scattering a full ration at once in the litter material resulted in increased foraging behaviour in P2 compared to feeding the larvae in a dedicated feeding trough. It would seem that the argument made by Pichova et al. (2016) and Veldkamp & van Niekerk (2019), that a gradual provision method would promote foraging behaviour to a higher extent, is supported. However, seeing as hens in the Scatter treatment were shown to engage in foraging behaviour to a similar extent it might not be necessary to extend the provision as long as the larvae are scattered. When the larvae are scattered, hens are forced to search the litter area and it is not as clear, as in the Trough treatment, when the larvae portion have been entirely consumed.

A combination of the Bucket and Scatter may perhaps be even more beneficial than their separate use. Ipema et al. (2016) evaluated a method which can be described as a combination between the Bucket and Scatter treatment used in this study. As previously described, the method in their study included scattering different amounts (5% versus 10%) of larvae at two different intervals (2 versus 4 times/day). Thus, the method included both an element of an extended time period with the larvae being available for a longer time period and a scatter element. The study was however performed using broilers and data collection was therefore performed at a much younger age compared to this study. It did however present interesting results where several provisions tended to promote foraging behaviour to a higher extent as compared to fewer. Future studies, on these combined methods are therefore recommended in order to identify the most promising provision method for hens.

All hens were observed to forage more in the second and third period with both occurring after the provision of live BSF larvae. These results are also in line with the studies by Pichova et al. (2016) and Ipema et al. (2020) where foraging behaviour increased after a provision of meal worms and live BSF larvae compared to the time period before. The time slots chosen for the video cameras to record were fairly in line with the diurnal rhythm for foraging behaviour in hens, with more bouts of foraging occurring at dawn (or when the lights go on in the stable) and in the late afternoon (Jensen 2017). As lights went on at 04.00 in this study it is possible that the first bout of foraging was partially missed as videos started to record at 07.00. Given this, the difference between the periods might have been less significant if the provision of larvae were performed earlier.

As the video cameras did not record the entire active period it is impossible to know how long the apparent effect of a provision of larvae lasts, future studies would need to look at a larger part of the active hours to be able to evaluate this.

5.1.2. Active behaviour

The time spent engaging in active behaviours were significantly affected by an interaction between period and treatment with hens in the Bucket treatment being more active compared to the Trough treatment in the second period (P2). Meanwhile, hens in the Scatter treatment did not differ from either of the other treatments during the same period (P2). In general, fewer active behaviours were registered during the first period (P1) as compared to the other two periods. These results are similar to other studies where a peak in active behaviour was observed after a provision of meal worms (Pichova et al. 2016) and live BSF larvae (Ipema et al. 2020).

5.1.3. Feather pecking

The low occurrence of FP was expected as the stocking density was low and hens were still quite young by the end of the study, and while gentle FP tends to decrease somewhat with age, severe FP will increase markedly as hens get older (Lambton et al. 2013).

While scoring the recordings in the present study, it was not uncommon to observe hens in the Bucket treatment “guarding” the area under the buckets as they noticed where the larvae would drop. Other studies have speculated that similar situations, where hens guarded an operant feeder and thus hindered others from eating may induce frustration in hens (Lindberg & Nicol 2001). However, due to the low occurrence of FP in the present study it is not possible to confirm if the hens experienced frustration due to the slow rate of larvae delivery in the Bucket treatment.

Studies have shown that FP among younger hens correlates with more FP later in life (Lambton et al. 2013) which is why even a low prevalence should be noted and discussed whilst evaluating methods to provide larvae. In this study, hens were kept at a much lower stocking density than that which is normally used in commercial stables and any differences may increase further under commercial conditions. Preferably, a follow up study examining any of the three methods included in this study would include a larger part of the laying period and commercial conditions (stocking density etc.) to provide a more realistic picture of a certain methods impact for farmers with regards to FP.

5.1.4. Agonistic behaviour

The low occurrence of agonistic behaviour was also expected as the use of EE has been shown to reduce the prevalence of aggressive behaviour such as aggressive pecking in hens (Johannson et al. 2016; Veldkamp & van Niekerk 2019). Furthermore, a low stocking density in combination with EE have yielded similar results (Zepp et al. 2018).

However, previous studies have shown that unreachable but visible feed presented to hens will induce both frustration and increase the time spent engaging in active behaviour such as walking and pacing (Haskell et al. 2000). Based on this it could be speculated that the Bucket treatment could potentially lead to frustration as larvae drop on a very small and well defined area in the litter. However, he low incidence of agonistic behaviour in the current study does not seem to give support to this idea. As previously suggested for FP it would be necessary to study hens during a longer time period, under commercial conditions, with larger groups and increased stocking density that could lead to increased competition for the larvae, in order to be able to analyse a realistic level of agonistic behaviour for each method.

5.2. Feed consumption

There was no effect of treatment on feed consumption. It did however fluctuate quite a bit over the weeks, indicating that the weighing of the left-over feed which was performed on a weekly basis was affected by something more than just consumption. According to the personnel working with the hens, it is not uncommon with spillage as hens eat but their perception during this study was that there had been rather little of that. However, even a little spillage could have quite an impact given that there were such few individuals in each pen. Future studies may benefit from being able to weigh the spillage as well to produce more precise data on feed consumption.

Looking at the average feed consumption in this study (89.6g/hen/day) compared to values from the breeding company (95-102g/hen/day; Bovans 2021) it would seem that hens ate less than would have been expected under normal conditions, where no other feed in the form of live BSF larvae is available. As expected and shown in previous studies (Tahamtani et al. *submitted*), hens thereby retained parts of their nutritional requirement from the larvae and it may therefore be able to contribute both nutrition and stimuli in the form of an EE.

5.3. Egg production and egg weight

There was no effect of treatment on the egg production or egg weight. Both Star et al. (2020) and Tahamtani et al. (*submitted*) recently showed that production parameters such as laying percentage and egg weight is similar for hens provided with live BSF larvae as for control groups which did not receive any larvae. Star et al. (2020) provided less larvae (10% of daily feed intake) compared to this study while Tahamtani et al. (*submitted*) provided larvae amounts equivalent to 10% and 20% of the daily nutritional need (dry matter basis) and also ad libitum, without any effect. Thus, it would seem that these production parameters remain unaffected regardless of the amount of larvae that is provided whilst other feed is also available.

An unaffected egg production is vital as hens are kept with the purpose of producing eggs and it would not be reasonable to go forward and further study a method which would negatively affect the production. Seeing as both a provision (Star et al. 2020; Tahamtani et al. *submitted*) and the provision method, at least the ones studied here, have no effect on production it should make it less troublesome to adapt a method suitable for farmers.

5.4. Weight and post mortem assessment

Finally, the results from this study showed that there were no effect of treatment for body weight or weight of liver, proventriculus, gizzard and fat pad. Furthermore, there was no interaction effect between week of age and treatment demonstrating that the growth during the data collection period was unaffected by treatment. These results were expected as all treatments were administered the same amount of larvae and previous studies have shown that body weight is unaffected despite differences in active behaviour (Schütz & Jensen 2001). The average hen weight during the study was also in agreement with the expected weights provided by the breeding company (Bovans 2021).

There was however an individual variation with some hens weighing more and having larger fat pads than others. The live BSF larvae has a high fat content (Liu et al. 2017) and previous studies have shown that a single hen is fully capable of consuming up to 160g live BSF larvae per day (Tahamtani et al. *submitted*). This amount is well above the intended portion of 62.5g/hen that was provided in this study. As the individual consumption of concentrate feed and larvae were not monitored, it is impossible to know the exact intake for each hen. However, one can speculate that some individuals managed to consume more larvae than others which may have affected their body weight and the size of their fat pad.

Individual data on intake of larvae in group housed hens would be interesting to collect as it would tell us if this is a type of EE that the majority will make use of and not just the dominant ones. If not, it might as previously mentioned induce frustration in the individuals that are unable to consume the larvae while watching others do (Lindberg & Nicol 2001). Also it is unclear how a high consumption might impact the health of the hens. As the larvae has a high fat content it could arguably have a negative impact which makes it further interesting to monitor the intake.

6. Conclusion

Both Bucket and Scatter treatment promoted foraging to a higher extent compared to Trough treatment. Thus, the hypotheses are partially supported as both an extended provision of larvae as compared to providing it all at once and providing it over a large area versus a small resulted in higher levels of foraging. However, only the extended provision resulted in higher levels of active behaviour as compared to providing larvae all at once. Seeing as FP and agonistic behaviour were rarely observed, it is not possible to make any conclusions regarding these behaviours. To make sure that slow delivery methods used to provide larvae does not induce frustration under commercial production settings, studies with larger groups and increased stocking density are needed. None of the treatments effected the production parameters measured in this study. Based on the results presented in this thesis, the Bucket and Scatter methods to provide live BSF larvae can be used to increase foraging behaviour in laying hens.

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Populärvetenskaplig sammanfattning

Värphöns som inte får sitt naturliga behov att söka efter föda tillfredsställt hanterar ibland detta genom att börja picka på andra höns samt dra loss deras fjädrar. Fenomenet är vanligt förekommande i dagens värphönsproduktion där foder som regel finns tillgängligt över tiden och är ett stort djurvälfärdsproblem eftersom det kan vara smärtsamt för den höna som blir pickad på, samtidigt som hönan som pickar uppenbarligen inte erbjudits tillräckliga möjligheter att utföra ett naturligt beteende.

För att motverka detta problem har forskare försökt hitta olika sätt att tillgodose behovet av att picka och sprätta genom att placera ut diverse ätbara ting såsom sallad, fröer, hela havrekärnor och jordnötssmör i värphönsstallar. Möjligheten att använda sig av insekter har också börjat undersökas med lovande resultat. Störst fokus ligger just nu på en insekt vid namn Svart soldatfluga och framförallt dennes larver.

Nyligen genomfördes en studie där födosöksbeteende hos höns med tillgång till levande larver undersöktes. I studien ingick totalt 90 värphöns som delades upp i tre grupper. Grupperna fick samma mängd larver men själva metoden för hur de tilldelades skiljde sig åt. I grupp 1 placerades larver i två upphängda spänner försedda med hål varpå larver föll ner bland hönsen under en längre tidsperiod, i grupp 2 spreds larver på en bädd av sågspån och grupp 3 fick sina larver serverade i två foderträg. Beteenden innan, i samband med, samt 5 timmar efter att hönsen fick tillgång till larverna spelades in med hjälp av videokameror och analyserades i efterhand. I övrigt studerades även en del produktionsvariabler (äggproduktion, foderkonsumtion och kroppsvikt) samt vikt på värphönsens inälvor.

Resultaten från studien visade att värphöns spenderar mer tid till att söka efter föda efter de fått tillgång till larver jämfört med innan. Vidare fanns skillnader mellan de olika tilldelningsmetoderna där höns i grupp 1 och 2 födosökte mer jämfört med höns i grupp 3. Hönsen i grupp 1 var också mer aktiva. Inga skillnader återfanns vad gällde de kontrollerade produktionsvariablerna.

En tilldelning av levande larver av Svart soldatfluga verkar därmed kunna främja födosöksbeteende hos värphöns utan att negativt påverka produktionen. Störst positiv effekt ses när larverna är tillgängliga under en längre tidsperiod (grupp 1) samt när de sprids ut över en större yta (grupp 2). Vidare studier, genomförda under de förhållande som återfinns i kommersiella värphönsstall krävs dock för att säkerställa att de positiva effekterna kvarstår vid implementering i större stall.